DNA double-strand breaks and ATM activation by transcription-blocking DNA lesions

Olivier Sordet[†], **Asako J. Nakamura**, **Christophe E. Redon**, **Yves Pommier**[*]

Laboratory of Molecular Pharmacology; National Cancer Institute; National Institutes of Health; Bethesda, MD USA

Abstract

Ataxia telangiectasia mutated (ATM), the deficiency of which causes a severe neurodegenerative disease, is a crucial mediator for the DNA double-strand break (DSB) response. We recently showed that transcription-blocking topoisomerase I cleavage complexes (TOP1cc) produce DSBs related to R-loop formation and activate ATM in post-mitotic neurons and lymphocytes. Here we discuss how TOP1cc can produce transcription arrest with R-loop formation and generate DSBs that activate ATM, as well as data suggesting that those transcription-dependent DSBs tend to form at the IgH locus and at specific genomic sites. We also address the potential roles of ATM in response to transcription-blocking TOP1cc.

Keywords

Introduction

DNA double-strand breaks (DSBs) are among the most severe genomic lesions, and their repair requires the recruitment of DNA damage response (DDR) proteins. The loss of function of DDR proteins leads to genomic instability and human hereditary diseases such as ataxia telangiectasia (AT caused by ataxia telangiectasia mutated (ATM) deficiency).[1] The gene product of ATM is a serine/threonine protein kinase activated by DSBs,[2] which phosphorylates various DDR proteins in the vicinity of damaged sites including histone H2AX, checkpoint kinase 2 (Chk2), mediator of DNA damage checkpoint 1 (MDC1) and p53 binding protein 1 (53BP1).[3] Phosphorylation of these proteins by ATM is critical for efficient DDR.[3] DSBs can be produced directly by ionizing radiation and radiomimetic molecules or indirectly during replication of damaged DNA by DNA polymerase collisions or replication fork collapse.[4] Until recently, it was not known whether transcription of damaged DNA templates could also lead to DSBs with ATM activation. This has been a longstanding concern as the main clinical feature of patients with AT is the acquisition of neuronal degeneration after full (post-replicative) development of the nervous system.[1]

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[*]Correspondence to: Yves Pommier; pommier@nih.gov.

[†]Present address: INSERM U563; Institut Claudius Regaud; Toulouse, France

Topoisomerase I (TOP1) removes DNA supercoiling generated by transcription by producing transient TOP1 cleavage complexes (TOP1cc), which are TOP1-linked DNA single-strand breaks.[5,6] The rapid resealing of TOP1cc is inhibited by common DNA base alterations[7] and by camptothecin (CPT) and its semi-synthetic derivatives topotecan and irinotecan, which are used to treat human cancers.[8] Stabilized TOP1cc are potent transcription-blocking DNA lesions.[9–11] Transcription complexes may be blocked physically a few base pair upstream of the TOP1cc[12,13] by the accumulation of positive DNA supercoiling ahead of the transcribing RNA polymerase II (Pol II).[14] Defective repair of transcription-blocking TOP1cc by inactivating mutation of tyrosyl-DNA-phosphodiesterase 1 (TDP1)[15,16] can cause spinocerebellar ataxia with axonal neuropathy (SCAN), an autosomal recessive neurodegenerative syndrome.[17] In our recent work, we used CPT to induce TOP1cc in non-replicating cells and determined whether transcription-blocking TOP1cc induce DSBs and activate the ATM-associated DDR.[18]

Transcription-Induced DNA Double-Strand Breaks and ATM Activation

We showed the induction of DSBs and DDR activation in post-mitotic primary neurons and lymphocytes treated with CPT, with the formation of large nuclear DDR foci containing activated ATM, phosphorylated H2AX at Serine 139 (γ-H2AX), activated Chk2, MDC1 and 53BP1.[18] A single DDR focus reflects hundreds to thousands of DDR proteins concentrated around at least one DSB.[4] The induction of γ-H2AX foci in neurons treated with CPT has simultaneously been reported by another group[19] and can be also detected in the non-S phase population of replicating HeLa cells treated with CPT.[18] Thus, stabilized TOP1cc produce replication-independent DSBs with activation of the ATM-associated DDR, asides from the well-established replication-associated DSBs in proliferating cells.[20]

Blocking Pol II by TOP1cc is likely the initial event for DSB production as selective inhibition of Pol II before the induction of TOP1cc suppresses DSB induction and ATM activation in post-mitotic cells as well as in the non-S phase population of replicating cells.[18] Further support for the occurrence of transcription-dependent DSBs is provided by colocalization experiments showing the localization of the γ-H2AX foci in euchromatin regions at sites where transcribing Pol II is arrested by TOP1cc.[18]

How could blocking elongating Pol II by TOP1cc induce DSBs? R-loops (RNA: DNA hybrids) are known to induce DSBs and genomic instability.[21,22] R-loops result from the extended pairing of nascent mRNA with the corresponding unwound DNA template behind the elongating Pol II.[22] We have shown that γ-H2AX signals are reduced by RNase HI overexpression, which removes transcription-mediated R-loops, in both post-mitotic neurons treated with CPT as well as in HeLa cells in which replication was blocked before treatment with CPT.[18] Thus, we proposed that R-loops generated by stalled TOP1cc induce the formation of DSBs (Fig. 1).[18] R-loops may form as negative supercoiling accumulates behind the transcription complexes arrested by TOP1cc.[23] Inhibition of the SR-kinase activity of TOPI by CPT[24] may also promote R-loops by interfering with splicing.[21] Although it is unclear how R-loops promote DSBs, CPT-induced R-loop-dependent DSBs may form in the 5' region of actively transcribing genes as TOP1cc arrest Pol II early in transcription[9,25] and DSBs seem to be produced within the region that forms R-loops.[21]

We have also detected transcription-induced DSBs in response to the transcription-coupled nucleotide excision repair (TCR) poison, ecteinascidin (Et-743) although those DSBs are dependent of the TCR and the MRE11-RAD50-NBS1 (MRN) complex.[26] Likewise, the histone deacetylase inhibitor, SAHA also induces transcription-dependent DSBs (Conti and Pommier, submitted). Hence, the formation of DSBs appears as a general response to DNA lesions and chromatin modifications that interfere with the translocation of Pol II through expressed genes.

Enrichment of Transcription-Dependent DSBs at Specific Genomic Regions

Transcription-dependent DDR foci score low as compared to replication-dependent DDR foci. An average of 2 and 5 γ-H2AX foci form per nucleus in response to CPT in post-mitotic lymphocytes and neurons, respectively.[18] Under similar conditions, multiple γ-H2AX foci (at least 50) form in the nuclei of replicating cells.[4,18,20] The low frequency of transcription-dependent γ-H2AX foci may reflect the induction of DSBs only at few specific genomic sites such as the transcribed regions prone to form R-loops.

R-loops tend to form at sequences that generate a G-rich transcript,[27–29] and such sequences are found at the mammalian immunoglobulin heavy (IgH) class switch regions.[29] Class switch recombination (CSR) is a recombination process that enables mammalian B-cells to generate antibody isotypes. CSR requires activation-induced cytidine deaminase (AID), an enzyme that requires DNA to be single-stranded and involves transcription and the generation of and R-loops at switch regions (reviewed in ref. 30). The induction of DSBs at switch regions has been visualized by the formation of γ-H2AX foci at the IgH locus in B cells activated for CSR.[31]

Owing to the susceptibility of IgH locus to form R-loops, we tested whether the DSBs induced by TOP1cc were enriched at that locus. We used immunocytochemistry staining followed by fluorescence in situ hybridization (ICC-FISH) to simultaneously visualize DSBs (detected by γ-H2AX foci) and specific DNA regions (IgH) in post-mitotic cells treated with CPT. Figure 2 shows an enrichment of γ-H2AX foci associated with sites of IgH in unstimulated splenocytes treated by CPT indicating that transcription-associated DSBs induced by TOP1cc form at specific genomic sites.

ATM Response to Transcription-Induced DNA Double-Strand Breaks

In our recent study,[18] we concluded that ATM is the main transducer of transcription-dependent DSBs in post-mitotic cells treated with CPT. Although some ATM substrates can also be phosphorylated by ataxia telangiectasia and Rad3 related (ATR)[32] and DNA-dependent protein kinase (DNA-PK),[33] ATR is not or only weakly expressed in post-mitotic lymphocytes[18,34] and neurons, respectively.[18,35] Also, CPT-induced γ-H2AX foci formation was unaffected by DNA-PK kinase inhibition in post-mitotic cells.[18] This is in marked contrast with replication-associated DSBs produced in CPT-treated proliferating cells where γ-H2AX is primarily induced by ATR and DNA-PK and in a lesser extend by ATM.[20]

It is well established that ATM signals DSB repair.[3] However, ATM seems primarily involved in the repair of DSBs in heterochromatin regions[36] whereas transcription-

associated DSBs form in euchromatin.[18] We have shown that ATM activates DNA-PK in post-mitotic cells treated by CPT.[18] Although the DNA-PK-dependent nonhomologous end-joining (NHEJ) pathway is considered to be the prevalent pathway for DSB repair in G_0 and G_1 leading to cell death phases of the cell cycle,[37] DNA-PK does not seem to be involved in the repair of transcription-associated DSBs in post-mitotic cells exposed to CPT.[18] Thus, other pathways are likely involved in repairing the transcription-associated DSBs in post-mitotic cells. Other potential roles of ATM activated by transcription-dependent DSBs include ROS quenching[38] and cell cycle re-entry of post-mitotic neurons leading to cell death[19,39] (Fig. 1). As detailed below, recent data suggest that ATM may also serve to repair or remove transcription-blocking TOP1cc as well as to prevent cumulative DNA lesions by inhibiting transcription (Fig. 1).

Transcription-blocking TOP1cc seem primarily removed by the TDP1 excision pathway. We refer the reader to recent reviews for a full description of TOP1 repair pathways.[40,41] Briefly, TOP1 excision by TDP1 requires prior proteolysis of TOP1. Our group and others have reported that TOP1 ubiquitination and subsequent degradation by the 26 S proteasome is selectively transcription dependent following CPT exposure,[25,42,43] as well as that TDP1 primarily repairs transcription-blocking TOP1cc as compared to replication-blocking TOP1cc.[15,16] Consistent with the involvement of the TDP1 pathway in the removal of transcription-blocking TOP1cc, TOP1 degradation has been suggested to promote resumption of RNA synthesis and cell survival.[43] Our recent observations suggest that ATM could activate the TDP1 excision pathway. First, TOP1 degradation is dependent of the DDR protein, BRCA1.[25] BRCA1 is a known ATM substrate,[44] although it is still unclear whether the ATM-dependent phosphorylation of BRCA1 is involved in TOP1 degradation. Second, TDP1 is phosphorylated/activated by ATM in response to CPT.[45] Thus, a working model is that TOP1cc produce transcription arrest and generate DSBs that activate ATM, which in turn removes transcription-blocking TOP1cc by promoting TOP1 degradation and TDP1 action. Based on this model, ATM might serve to promote resumption of RNA synthesis and cell survival following transcription arrest by TOP1cc.

The ATM pathway has been shown to inhibit Pol I transcription in response to DSBs.[46] These findings and our work showing the activation of ATM by transcription-induced DSBs,[18] suggest the existence of an ATM-dependent transcription checkpoint pathway, in which few localized transcription-associated DSBs activate ATM, which in turn inhibits transcription more globally. In line with this hypothesis, approximately 80 to 90% of transcription is inhibited within minutes following CPT exposure,[43] and some ATM substrates are involved in transcription regulation such as the corepressor of gene transcription Kap-1,[47] and the transcription factor Spl.[48] Thus, similar to replication checkpoint that arrest DNA replication and cell cycle progression, global transcription arrest could provide time for cells to repair the transcription-associated DNA lesions and thereby to prevent additional genomic alterations resulting from transcription of a damaged DNA template.

Conclusions and Perspectives

The transcription- and R-loop-dependent DSBs, which are potentially lethal if not repaired, might contribute to the cytotoxicity of TOP1cc in post-mitotic cells.[19,39,49] Thus, defective response to TOP1-induced transcription-dependent DSBs could be involved in the death of neurons in AT and SCAN patients. As should be the case for novel insights, our findings raise a set of new inquiries. First, it will be important to know whether stabilization of TOP1cc by common DNA lesions (i.e., DNA oxidative lesions)[7,40] can also promote the induction of transcription-dependent DSBs with ATM activation. Other issues concern the IgH locus being targeted in lymphoid cells treated with CPT in the absence of immune stimulation and the involvement of AID in the formation of the transcription-induced DSBs by TOP1cc. Finally, it will be relevant to know the distribution of the transcription-dependent DSBs across the genome and whether genome-wide analyses will identify hot-spot regions where transcription-associated DSBs are produced in response to CPT.

Acknowledgements

Abbreviations:

AID	activation-induced cytidine deaminase
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia and Rad3 related
Chk2	checkpoint kinase 2
CPT	camptothecin
DDR	DNA damage response
DNA-PK	DNA-dependent protein kinase
CSR	class switch recombination
DSB	DNA double-strand break
γ-H2AX	phosphorylated histone H2AX on Ser139
MDC1	mediator of DNA damage checkpoint 1
53BP1	p53 binding protein 1
Pol II	RNA polymerase II
SCAN	spinocerebellar ataxia with axonal neuropathy
TCR	transcription-coupled nucleotide excision repair

TDP1	tyrosyl-DNA-phosphodiesterase 1
TOP1	topoisomerase I
TOP1cc	topoisomerase I cleavage complexes

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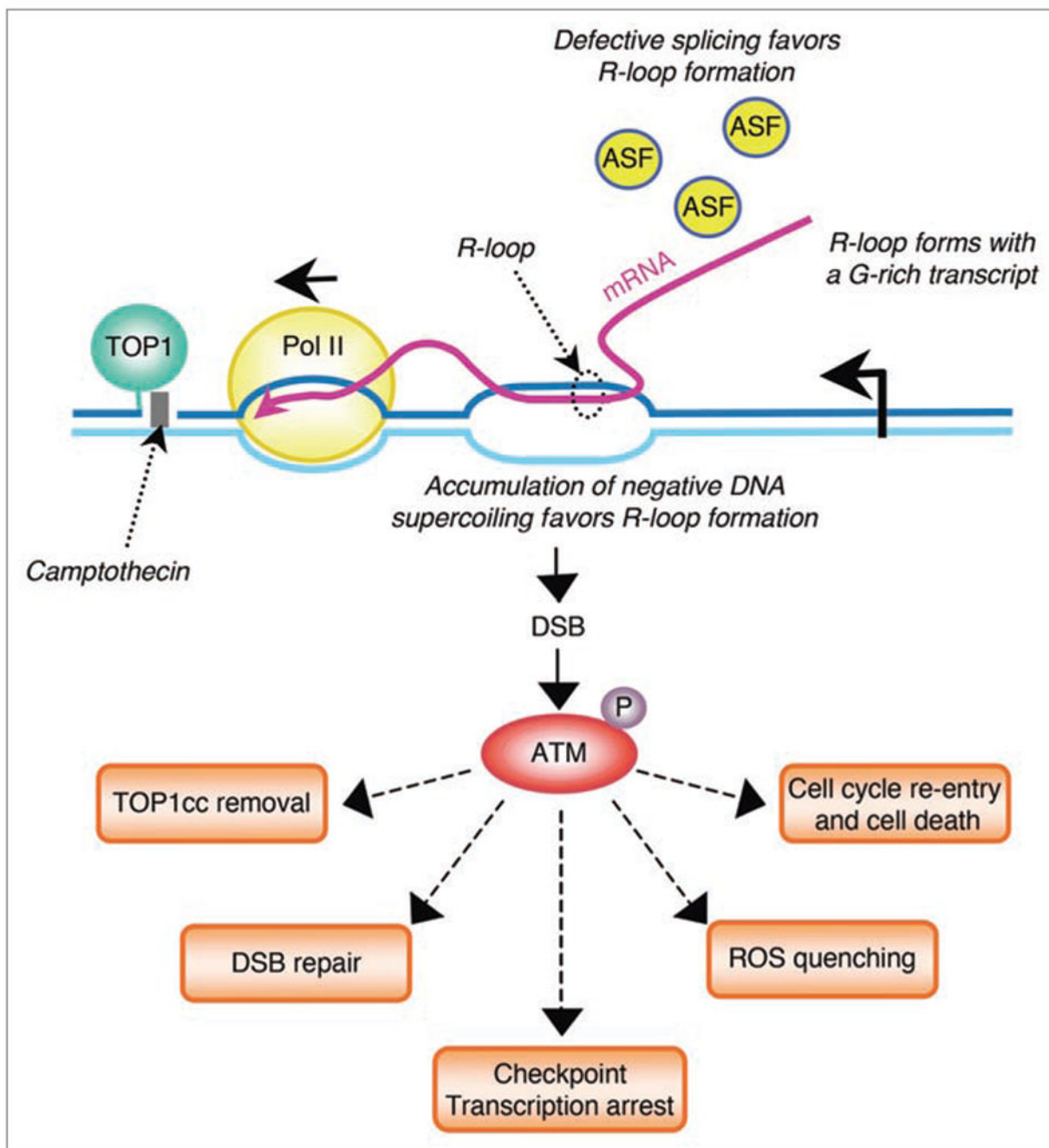
Figure 1.
Potential roles of ATM activated by transcription- and TOP1cc-induced DSBs. Stabilization of TOP1cc by CPT interferes with transcription elongation and generates a hybrid RNA-DNA (R-loop). R-loops may form as negative supercoiling accumulates behind the transcription complexes arrested by TOP1cc. Inhibition of TOPI SR-kinase activity by CPT may also promote R-loops by interfering with splicing because of alternative splicing factor (ASF) hypophosphorylation. R-loops form with a G-rich transcript. R-loop promotes the induction of a DSB, which leads to autophosphorylation/activation of ATM in the vicinity of

the DSB. ATM may promote TOP1cc removal, DSB repair, transcription arrest and ROS quenching. Besides its protective role, ATM may also promote cell cycle re-entry of post-mitotic neurons leading to neuronal death.
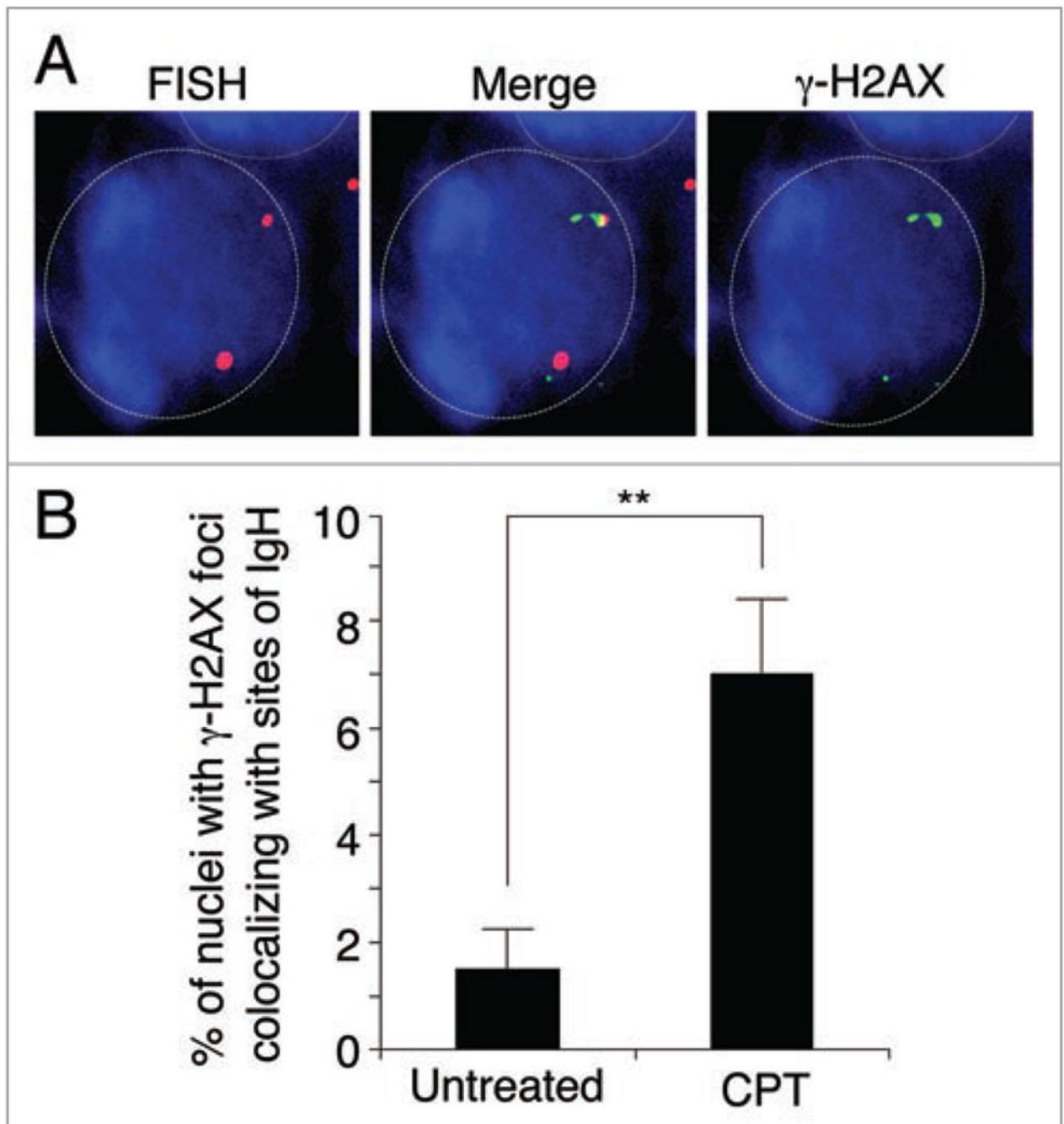
Figure 2.
Colocalization of γ-H2AX and IgH increases after CPT treatment in post-mitotic splenocytes. (A) Representative image of immunostaining for γ-H2AX (green) and fluorescent in situ hybridization (FISH) for IgH (red). DNA was counterstained with DAPI (blue). Nuclear outlines are highlighted. Mouse splenocytes were treated with 25 μM CPT for 1 h and slides were prepared as described previously.[31] (B) Percentage of cells in which γ-H2AX and IgH colocalized. The data shows the average of two independent experiments in which 100 cells were counted for each experiment. Error bars signify standard deviation.

Statistical analysis was carried out using Fishers exact test. A double asterisk indicates a p-value <0.005.